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Reproductive Dysfunction in Women with Albright's Hereditary Osteodystrophy*

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ABSTRACT

Most individuals with Albright's hereditary osteodystrophy (AHO) have deficient expression or function of $G_{s\alpha}$, the alpha subunit of the guanine nucleotide binding protein that stimulates adenylyl cyclase, and are resistant to parathyroid hormone (PTH) and other hormones that act via stimulation of adenylyl cyclase. To determine the incidence and etiology of ovarian dysfunction in women with AHO, we examined the reproductive history and hypothalamic-pituitary-ovarian axis in 17 affected women aged 17–43 yr. All patients had typical PTH resistance and an approximately 50% reduction in erythrocyte $G_{s\alpha}$ activity. (0.43 ± 0.03 *vs.* 0.92 ± 0.08 for normal control subjects, $P < 0.001$). Fourteen of the 17 patients (76%) were oligomenorrheic or amenorrheic, more than half had delayed or incomplete sexual development, and only two had a history of earlier pregnancy. Most women were mildly hypoestrogenic, with normal to slightly elevated serum gonadotropin levels. Computer analysis of 24° LH measurement showed that the frequency of LH peaks/24 h in AHO women varied widely, but as a group they were not statistically different from a group of normal women studied in the early follicular phase. Administration of 100 μg synthetic GnRH produced normal FSH and LH responses. We conclude that reproductive dysfunction is common in women with AHO and probably represents partial resistance to gonadotropins. (*J Clin Endocrinol Metab* **83:** 824–829,1998)

ALBRIGHT'S hereditary osteodystrophy (AHO) is an inherited metabolic disorder characterized by an unusual constellation of somatic and developmental defects, including short stature, brachydactyly, obesity, subcutaneous ossifications (1), and deficient expression or function of the α subunit of the guanine nucleotide regulatory protein ($G_{s\alpha}$) that stimulates adenylyl cyclase (2, 3). In addition, most patients with AHO show resistance to multiple hormones (*e.g.* parathyroid hormone, thyroid stimulating hormone, and glucagon) that bind to receptors that require $G_{s\alpha}$ for activation of adenylyl cyclase (AC). This condition has been termed pseudohypoparathyroidism type 1a (PHP 1a) (4). By contrast, some affected subjects appear to have normal endocrine responsiveness despite $G_{s\alpha}$ deficiency, a condition that has been termed pseudopseudohypoparathyroidism (PPHP) (5).

Although several case reports and clinical studies (4, 6, 7) have alluded to menstrual irregularities and hypogonadism in women with AHO, the cause and significance of female reproductive dysfunction in AHO remains unknown. If the basis of hypogonadism is ovarian resistance to stimulation by follicle stimulating hormone (FSH) and luteinizing hormone (LH), similar to the target tissue resistance to PTH and TSH, one would expect the clinical picture of hypogonadism to be accompanied by elevated levels of plasma gonadotropins. This mechanism is supported by the description by Wolfsdorf *et al.* (7) of a woman with AHO and PHP type 1a who was oligomenorrheic and had elevated basal levels of gonadotropins. By contrast, other reports have described women with AHO who have ovarian dysfunction and apparently normal gonadotropin levels (8). To determine the incidence, mechanism, and natural history of reproductive dysfunction in women with AHO, we evaluated the reproductive history and hypothalamic-pituitary-ovarian axis in women with AHO and PHP type 1a.

Subjects and Methods

Patients

Seventeen women (aged 17–43 yr) with PHP type 1a were evaluated, but some women did not undergo all tests. All met criteria for AHO including brachydactyly, unilateral or bilateral, involving hands or feet, short stature, and decreased $G_{s\alpha}$ activity. Subcutaneous ossifications were present in 11 of the women. All of the women were normocalcemic on vitamin D and calcium supplementation, and all were euthyroid at the time of testing. The control group consisted of 13 normal adult women who had regular menstrual cycles. Menstrual histories, Tanner developmental stages, and peripheral blood cell karyotypes were obtained. Body mass index (BMI) was derived from measurements of height and weight. Clinical information including menstrual history, pregnancy history, and history of hormonal use was obtained for the period of time up to 10 yr after the initial studies. Written informed consent was obtained from all patients and controls.

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Hormone assays

Basal plasma levels of reproductive hormones were determined by radioimmunoassay (Hazelton Laboratories, Vienna, VA), including estradiol (E_2), progesterone (P), prolactin, LH, FSH, 17-hydroxy progesterone, testosterone, androstenedione, and dehydroepiandrosterone. In women with regular menses, blood samples were obtained during the early follicular phase of the cycle. As it was not possible to determine the phase of the cycle in oligomenorrheic or amenorrheic women, blood samples were drawn randomly in these women. In some women, pelvic ultrasonographic examination was performed to evaluate ovarian structure and size.

LH pulsatility

Eleven women had blood samples drawn from an indwelling catheter at 20-min intervals during a 24-h period for gonadotropin measurement and analysis of LH pulsatility. Testing was conducted at the Clinical Center of the National Institutes of Health. The pattern of LH peaks was analyzed with the Pulsar program (Pharmacia Peptide Hormones, Stockholm, Sweden), which scores LH peaks by height and duration from a smoothed local baseline, using the dose-dependent radioimmunoassay variance as a scale factor (9, 10). For these studies cutoffs were 3.8, 2.6, 1.9, 1.5, and 1.2 SD for peaks 1–5 points in duration, respectively. The intrassay coefficient of variation for LH measurements averaged 5.3% over the range of values studied. Results were compared with those obtained from a group of normally cycling women sampled at the same frequency who were studied in the early- to mid-follicular phase.

Provocative tests

Eight patients underwent gonadotropin releasing hormone (GnRH) stimulation tests with 100 μg synthetic GnRH. Samples were drawn at 0, 15, 30, 60, and 120 min for measurement of LH and FSH levels. In some patients, progestin-induced withdrawal bleeding was tested by the administration of a single intramuscular injection of progesterone in oil (200 mg). In other patients, medroxyprogesterone (5 mg) was given daily for the last 10 days of a month in which they also took estrogen (*e.g.* conjugated equine estrogens 0.625 mg) daily. The onset of uterine bleeding within 10 days of administration of progestin was considered evidence of a positive response.

Determination of erythrocyte $G_{s\alpha}$ Activity

The biological activity of $G_{s\alpha}$ was determined using a complementation assay (3, 11) based on the ability of solubilized extracts of erythrocyte membranes to reconstitute the responsiveness of adenylyl cyclase in membranes prepared from S49 cyc^- murine lymphoma cells, which genetically lack $G_{s\alpha}$ protein (12). Blood was obtained from patients and from control subjects by venipuncture and anticoagulated with acid citrate dextrose. Erythrocyte membranes were prepared as previously described (3), and soluble extracts were obtained by treatment with 0.2% (wt/vol) Lubrol PX (ICN Biomedicals, Aurora, OH).

Soluble extracts were assayed for $G_{s\alpha}$ activity using S49 cyc^- membranes, essentially as previously described (13). The assay for $G_{s\alpha}$ activity is linear with respect to the amount of extract protein added. Results of assays were expressed as a percentage of the activity of a standard membrane preparation consisting of pooled erythrocytes from five normal persons, and they represent the mean of at least three separate determinations.

Ovarian histology and $G_{s\alpha}$ messenger RNA (mRNA) analysis

One patient who underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy for unrelated reasons had ovarian tissue submitted for histological evaluation and $G_{s\alpha}$ mRNA analysis. Total cellular RNA was isolated from ovarian tissue by the guanidinium isothiocyanate-cesium chloride technique (14). First-strand complementary DNA (cDNA) was synthesized from 5 μg RNA in a 20-μL reaction mixture containing 100 pmol of random hexamer primers and 200 units Moloney murine leukemia virus reverse transcriptase (BRL, Gaithersburg, MD) (15). Aliquots of cDNA served as templates for *in vitro* amplification by PCR (16), in patient 5, of a 600 bp fragment of $G\alpha_s$ cDNA using synthetic oligonucleotides that flank the known GNAS1 missense mutation (R165C) (17). The primers were designed using the MELTMAP 87 program (generously provided by Dr. L. S. Lerman, MIT, Cambridge, MA) to optimize analysis by denaturing gel electrophoresis (DGGE); to increase the sensitivity of DGGE one oligonucleotide of the pair was synthesized with a 5′ GC-rich clamp. Oligonucleotide primers were synthesized by phosphoramidite chemistry, using a Milligen/Biosearch Cyclone Plus DNA Synthesizer (Burlington, MA). Reactions contained 3 μL first-strand cDNA in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.5 μM of each primer(sense primer located in exon 5, 5′GACTTCCCTCCCGAATTCTATGAG; antisense primer located in exon 9, 5′*GCGCCCGGCGCCGCCCGCCGGCGCGGCCCGCCCGCGGG*-CGAAGCACTGGATCCACTTGCGGCG), 0.25 mM each dATP, dCTP, dGTP, dTTP, and 2.0 units of *Taq* polymerase (Perkin Elmer-Cetus Corp., Norwalk, CT). After an initial denaturation for 4 min at 94 C, the samples underwent 40 amplification cycles consisting of denaturation for 1 min at 94 C, annealing for 1 min at 55 C, and extension for 2 min at 72 C, with a final extension of 10 min.

Amplified DNA samples were analyzed by DGGE as previously described (17, 18). Briefly, samples (15–30 μL) were electrophoresed at 60 C for 16 h at 85 volts in 6.5% polyacrylamide gels containing a denaturant gradient (40–80%) parallel to the direction of electrophoresis (100% denaturant = 7 M urea and 40% formamide). After electrophoresis, gels were stained with ethidium bromide (1 μg/mL) and photographed by UV transillumination with Polaroid type-55 film (Rochester, NY).

Statistical analysis

Data were analyzed with the Student *t* test for unpaired samples. The 95% confidence interval was used for testing significance. Unless otherwise stated, results are expressed as mean $\pm$ 1 SD.

Results

$G_{s\alpha}$ levels and clinical profiles

Patients with PHP type 1a had a reduction of approximately 50% in erythrocyte $G_{s\alpha}$ activity (0.43 $\pm$ 0.03 *vs.* 0.92 $\pm$ 0.08 $P < 0.001$) (Table 1). There was no relationship between the level of $G_{s\alpha}$ activity and any clinical or reproductive endocrine parameters.

Menstrual histories of the 17 women with AHO and PHP type 1a were obtained (Table 2). Four of these women had regular menses, 4 had primary amenorrhea, 1 had secondary amenorrhea, and 8 had oligomenorrhea. Two of the women with oligomenorrhea had prior pregnancies. Physical examination revealed incomplete development of secondary sexual characteristics (Tanner III-IV) in the majority of patients. Mild hirsutism was noted in 3 patients. The mean height was 145 cm, the mean weight was 66.6 kg, and the mean BMI was 31.7.

Karyotypes were normal (46XX) in all patients except patient number 12, who had a chromosomal translocation (46XXt) (13–15). Pelvic ultrasonography was performed in 9 cases and showed small- to normal-sized ovaries in all patients examined. Small follicular cysts, evidence of follicular activity, were noted in only 5 of the 9 patients. Failure to observe follicular activity in the remaining patients may have been the result of random timing of ultrasonography in the oligomenorrheic patients.

Basal hormonal profile

Mean basal hormone levels in the PHP type 1a patients are presented in Table 1. Plasma estradiol levels were less than 50 pg/mL in 14 of the 17 women, similar to levels present

TABLE 1. Hormonal function in AHO females

Patient no.	E_2 (pg/mL)	Progesterone (ng/mL)	Mean LH (IU/L)	Mean FSH (IU/L)	Prolactin (μg/L)	LH peaks/24 h	Erythrocyte $G_{s\alpha}$
1	39	0.10	21	17	12	7	0.41
2	40	0.35	17	14	21	15	0.66
3	30	0.30	11	9	8	16	0.52
4	28	0.12	28	48	4	10	0.45
5	34	0.28	8	21	9	11	0.36
6	71	0.14	12	17	4	11	0.36
7	35	0.13	9	16	3	11	0.39
8	40	0.18	15	20	3	11	0.47
9	30	0.28	13	13	3	12	0.54
10	159	0.75	39	25	16	18	0.68
11	10	0.20	15	18	3	2	0.51
12	16	0.15	15	22	8		0.22
13	66	0.16	41	22			0.49
14	28		28	8	38		0.47
15	30		10	8	20		0.40
16	10						0.53
17	29		14	16	3		0.58
Mean ± SD	40.9 ± 34.4	0.24 ± 0.17	18.8 ± 10.5	18.4 ± 9.4	10.3 ± 9.9	11.2 ± 4.3	0.47 ± 0.11
Normal follicular range	12–100	0.1–1.1	6–27	7–27	2–37	12 + 5.2[a]	0.92 + 0.08[a]

[a] Normal controls

TABLE 2. Clinical characteristics of patients studied

Patient	Age	Gravida-Para	Menstrual function	Breast Tanner stage
1	24	0–0	O	3–4
2	27	0–0	N	
3	17	0–0	N	4–5
4	43	3–1	N→O	5
5	22	0–0	O	3–4
6	24	1–1	O	5
7	18	0–0	1A	3
8	20	0–0	1A	2
9	23	0–0	O	3
10	23	0–0	2A	
11	33	0–0	O	3
12	25	0–0	1A	3–5
13	16	0–0	O	
14	24	0–0	N	5
15	24	0–0	N	5
16	36	0–0	O	
17	18	0–0	1A	3–5

Clinical status: O_2, Oligomenorrhea; 1A, Primary Amenorrhea; 2A, Secondary Amenorrhea; N, Normal Menstrual Periods.

during the normal early follicular phase. Plasma concentrations of progesterone were low, similar to levels present in the follicular phase, in all patients. Serum concentrations of gonadotropins FSH and LH were normal to slightly elevated. Prolactin levels were normal in all except patient number 14, whose prolactin level was minimally elevated. Serum concentrations of androgens were normal in all women tested.

Because of dysfunctional uterine bleeding, patient number 5 later underwent hysterectomy with bilateral salpingo-oophorectomy and subsequently developed markedly elevated levels of serum gonadotropins that were appropriate for a postmenopausal female.

LH Pulsatility

Pulsar analysis of plasma LH values obtained during the 24-h sampling (Table 1) showed that the frequency of LH peaks per 24° in the PHP type 1A group was not statistically different from that of a group of normal women studied in the early follicular phase. (11.8 ± 4.9 *vs.* 12 ± 5.2). Some women, however, had high (subjects 2, 3, and 10) or low (subjects 1 and 11) pulse frequencies.

GnRH stimulation tests

LH responses (Fig. 1) and FSH responses (Fig. 2) to GnRH stimulation were similar to controls, with the exception of patient number 4, who was perimenopausal. However, given that the AHO patients were hypoestrogenic at the time of testing, these responses may underestimate responsiveness of the pituitary to GnRH.

Ovarian histology and $G_{s\alpha}$ mRNA expression

Gross examination and histological analysis of the resected ovarian tissue from patient number 5 revealed multiple follicular cysts that measured up to 1 cm. Several atretic scars were present, but no corpora lutea were identified.

PCR-amplified $G_{s\alpha}$ cDNA synthesized from ovarian mRNA was analyzed by denaturing gradient gel electrophoresis and showed an abnormal pattern. In addition to a DNA fragment corresponding to a wild type $G_{s\alpha}$ allele, a more slowly migrating fragment that contained an R165C missense mutation (1072) was also observed (Fig. 3). The two, more slowly migrating DNA fragments represent heteroduplexes formed between normal and abnormal DNA strands during PCR.

Natural history

Long-term information on reproductive function was obtained from eight women, with a mean follow-up length of 8.6 yr. All women who had regular menses at the time of the initial study continued to have regular menses. Most women with 1° amenorrhea continued to be amenorrheic, but one woman reported the spontaneous onset of menses at age 28. Women with 2° amenorrhea and oligomenorrhea remained amenorrheic or had occasional spontaneous menses. None of these eight women had subsequent pregnancies despite un-

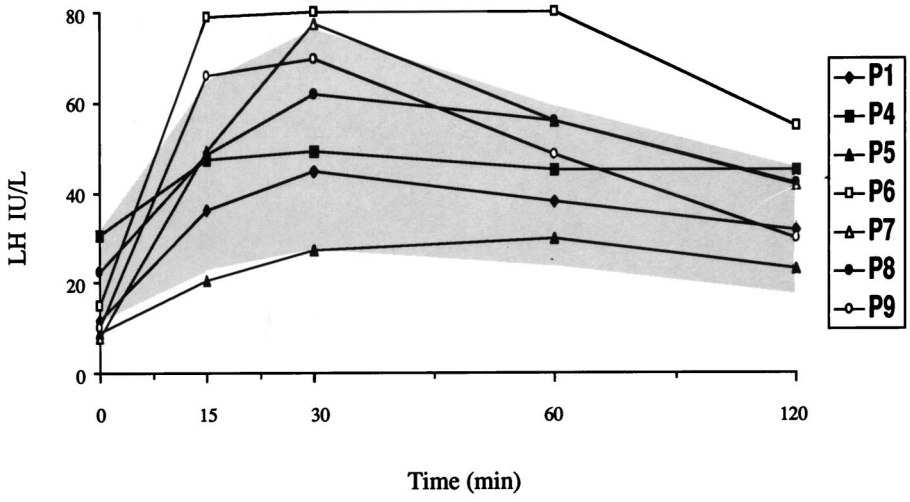

FIG. 1. Serum LH responses to administration of GnRH. Serum concentrations of LH are presented before and after the iv injection of 100 μg GnRH at time zero. The *shaded area* represents LH values corresponding to one standard deviation above and below the mean for normal women who were studied on day 5 of their menstrual cycle. Symbols denote values for patients described in the text.

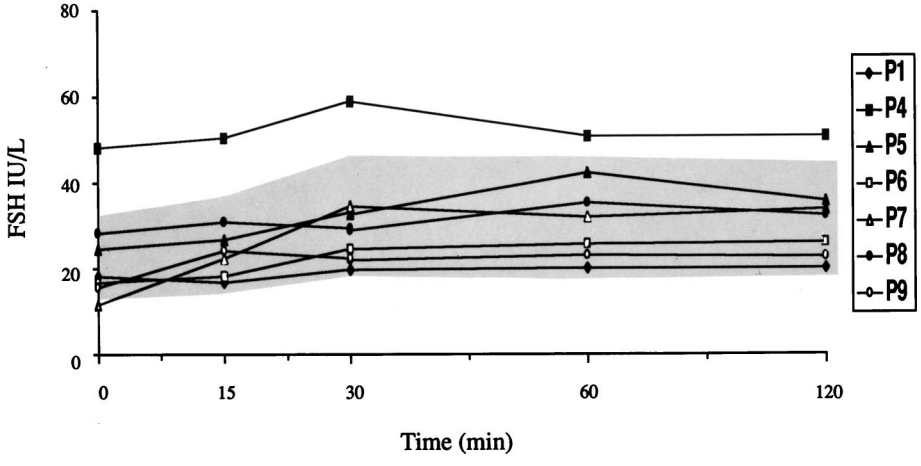

FIG. 2. Serum FSH responses to administration of GnRH. Serum concentrations of FSH are presented before and after the iv injection of 100 μg GnRH at time zero. The *shaded area* represents FSH values corresponding to one standard deviation above and below the mean for normal women who were studied on day 5 of their menstrual cycle. Symbols denote values for patients described in the text.

protected intercourse. Estrogen and/or progestin therapy was given to approximately half of the women. Most patients had a weak response or no response to progestin withdrawal unless estrogen was also given.

Discussion

Gonadal function is affected in several syndromes that involve abnormal function or activity of G proteins. In part, this may be due to the presence of multiple G protein-coupled signaling pathways that are involved in gonadotropin action, including both the adenylyl cyclase and the phospholipase C pathways (19–21). Children with McCune-Albright syndrome, a disorder in which somatic mutation of the GNAS1 gene results in mosaic distribution of cells containing an activated form of $G_{s\alpha}$ (22, 23), commonly have precocious puberty as well as autonomous hyperfunction of other endocrine glands (24). Iiri *et al*. (25) recently described two males with both precocious puberty and PHP type 1a. These two unrelated boys had identical GNAS1 gene mutations that resulted in a temperature-sensitive $G_{s\alpha}$ that is constitutively activated in the cooler environment of the testis, while being rapidly degraded in other tissues at normal body temperature. These studies indicate that gonadal function is highly influenced by the activity of $G_{s\alpha}$.

Deficient activity of $G_{s\alpha}$ also has profound effects on reproductive function. In our series, three fourths of the AHO females with $G_{s\alpha}$ deficiency (PHP type 1a) were oligomenorrheic or amenorrheic, and 9 out of 12 had delayed puberty or incomplete sexual development. Reproductive dysfunction in these patients was not complete, however; some women had normal menstrual cycles, and two women with irregular menses had had full-term pregnancies in the past.

If ovarian dysfunction occurs via a mechanism of hormone resistance that is similar to that which accounts for TSH and PTH resistance in the thyroid and kidney, respectively, decreased secretion of estrogen would be expected to be accompanied by elevated gonadotropin levels, as in the single AHO patient described by Wolfsdorf *et al*. (7), and subsequently shown by us to have $G_{s\alpha}$ deficiency (patient 2b, ref 26). Therefore, we were surprised that serum gonadotropin levels were either normal or only slightly elevated in the women in our study, despite the fact that most of them were hypoestrogenic, as confirmed by scant or absent withdrawal bleeding after progestin administration. Although these results are consistent with a pattern of chronic anovulation of central etiology, several lines of evidence argue against this mechanism as the primary basis for reproductive dysfunction. First, most patients showed normal or increased LH

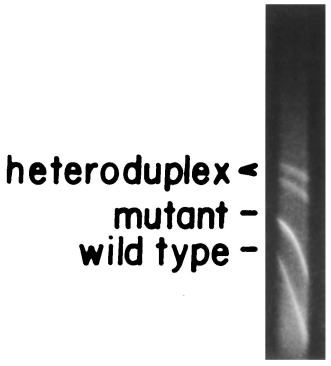

FIG. 3. Analysis of $G_{s\alpha}$ cDNA by denaturing gradient gel electrophoresis. An aliquot of total RNA isolated from ovarian tissue of patient 5 was reverse-transcribed, and the cDNA was amplified by PCR, as described in the text. The resulting DNA fragments were resolved by denaturing gradient gel electrophoresis, and the gel was stained with ethidium bromide. The two slowly migrating fragments represent heteroduplexes between the wild type and mutant (R165C) DNA fragments formed during the PCR; the mutant DNA homoduplex migrates more slowly than the wild-type DNA homoduplex.

pulsatility. Second, serum levels of gonadotropins were appropriately elevated in AHO females who were perimenopausal (patient 4) or postmenopausal (patient 5). Third, GnRH receptors are not directly coupled to the cAMP pathway, and thus a deficiency of $G_{s\alpha}$ should not have a significant effect on GnRH responsiveness. One mechanism to explain these clinical and biochemical findings is partial resistance of the theca and granulosa cells of the ovary to gonadotropins due to deficient $G_{s\alpha}$ activity. We found that one half of the $G_{s\alpha}$ mRNA in ovarian tissue from patient 5 was transcribed from the defective GNAS1 gene, consistent with a 50% reduction in levels of $G_{s\alpha}$ protein (not shown). Because both LH and FSH receptors are coupled to $G_{s\alpha}$ in the ovary, deficient expression or activity of $G_{s\alpha}$ might lead to a state of partial responsiveness to gonadotropins. Responsiveness might be sufficient to promote some degree of follicular development and steroid secretion, but might be insufficient to induce ovulation. Specifically, the estradiol levels may be adequate to exert negative feedback on gonadotropins (resulting in normal to slightly elevated gonadotropin levels), but may be inadequate to trigger the midcycle LH surge (*i.e.* positive feedback). Sonographic and histological findings of limited follicular development in these patients support this hypothesis.

Further evidence in support of the premise that partial ovarian resistance occurs in AHO is provided by the patient described by Wolfsdorf *et al*. (7), who was treated in order to induce ovulation. Administration of clomiphene citrate failed to induce ovulation, as might be expected in a hypoestrogenic patient. Despite elevated basal levels of FSH and LH, administration of human menopausal gonadotropins (hMG) stimulated an appropriate rise in serum estradiol to more than 500 pg/mL, indicating that, in at least some cases, partial ovarian resistance can be overcome by very high levels of gonadotropins. Despite the notable differences in basal gonadotropin levels between the patient described by Wolfsdorf *et al*. (7) and the patients we have described in this study, the similar molecular pathophysiology of $G_{s\alpha}$ deficiency in all these patients implicates a common mechanism of reproductive dysfunction.

Reduced expression or function of $G_{s\alpha}$ likely accounts for hormone resistance and reproductive dysfunction in women with PHP type 1a. By contrast, reproductive function is generally normal in women with pseudoPHP despite $G_{s\alpha}$ deficiency and GNAS1 mutations, which are indistinguishable from relatives with PHP type 1a. The basis for variable penetrance of hormone resistance in AHO is unknown. The observation that maternal transmission of $G_{s\alpha}$ deficiency leads to PHP type Ia, whereas paternal transmission of the defect leads to pseudoPHP (13, 27, 28), has implicated paternal imprinting as a possible explanation for the different phenotypes of identical GNAS1 gene defects (28, 29). Imprinting of this locus would be consistent with the chromosomal localization of GNAS1 at 20q13.11 (30), a region showing syntenic homology with the imprinted murine region 2E1–2H3 (31, 32). Indeed, recent studies have demonstrated genomic imprinting of the murine *Gnas* gene in fetal mouse tissues (33). Interestingly, both maternal and paternal *Gnas* alleles are expressed in a wide range of tissues, although only the paternal allele is expressed within the renal glomerulus (33). The restricted pattern of tissue- (or cell-) specific imprinting of the *Gnas* gene in murine embryos at late gestation is consistent with previous studies showing transcription of both GNAS1 gene alleles in tissues from human fetuses (34). In the present study we found that ovarian tissue from patient number 5 contained equivalent amounts of both wild type and mutant $G_{s\alpha}$ transcripts (Fig. 3), indicating than both GNAS alleles are expressed in the preponderant cell types, *i.e.* theca and granulosa cells, present in the adult ovary. These results support our clinical findings that levels of LH and FSH are not markedly elevated in women with AHO and are consistent with the hypothesis that ovarian resistance to gonadotropins is incomplete.

We conclude that reproductive dysfunction is common in women with PHP type 1a and likely involves partial resistance to gonadotropins in the granulosa and theca cells of the ovary. The resistance to gonadotropins in women with PHP type 1a is more subtle than the resistance that occurs to some hormones (*e.g.* PTH, TSH) but is more significant than the resistance that occurs to other hormones (*e.g.* glucagon, vasopressin). Further studies will likely reveal whether these differences in hormone responsiveness relate to cell-specific differences in the imprinting of the GNAS1 genes.

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